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Abstract: Patients in whom virologic suppression is achieved with highly active antiretroviral therapy (HAART) retain long-lived cellular reservoirs of human immunodeficiency virus type 1 (HIV-1); this retention is an obstacle to sustained control of infection. To assess the impact that initiating treatment during primary HIV-1 infection has on this cell population, we analyzed the decay kinetics of HIV-1 DNA and of infectivity associated with cells activated *ex vivo* in 27 patients who initiated therapy before or <6 months after seroconversion and in whom viremia was suppressed to <50 copies/mL. The clearance rates of cellular reservoirs could not be distinguished by these techniques (median half-life, 20 weeks) during the first year of HAART. The clearance of HIV-1 DNA slowed significantly during the subsequent 3 years of treatment (median half-life, 70 weeks), consistent with heterogeneous cellular reservoirs being present. Total cell-associated infectivity (CAI) after 1 year of treatment was undetectable (<0.07 infectious units/million cells [IUPM]) in most patients initiating treatment during primary infection either before (9/9) or <6 months after (6/8) seroconversion. In contrast, all 17 control patients who initiated HAART during chronic infection retained detectable CAI after 3-6 years of treatment (median reservoir size, 1.1 IUPM; $P < .0005$). These results suggest that treatment <6 months after seroconversion may facilitate long-term control of cellular reservoirs that maintain HIV-1 infection during treatment

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Effect of Treatment, during Primary Infection, on Establishment and Clearance of Cellular Reservoirs of HIV-1

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(See the editorial commentary by Blankson et al., on pages 1394–6.)

Patients in whom virologic suppression is achieved with highly active antiretroviral therapy (HAART) retain long-lived cellular reservoirs of human immunodeficiency virus type 1 (HIV-1); this retention is an obstacle to sustained control of infection. To assess the impact that initiating treatment during primary HIV-1 infection has on this cell population, we analyzed the decay kinetics of HIV-1 DNA and of infectivity associated with cells activated *ex vivo* in 27 patients who initiated therapy before or <6 months after seroconversion and in whom viremia was suppressed to <50 copies/mL. The clearance rates of cellular reservoirs could not be distinguished by these techniques (median half-life, 20 weeks) during the first year of HAART. The clearance of HIV-1 DNA slowed significantly during the subsequent 3 years of treatment (median half-life, 70 weeks), consistent with heterogeneous cellular reservoirs being present. Total cell-associated infectivity (CAI) after 1 year of treatment was undetectable (<0.07 infectious units/million cells [IUPM]) in most patients initiating treatment during primary infection either before (9/9) or <6 months after (6/8) seroconversion. In contrast, all 17 control patients who initiated HAART during chronic infection retained detectable CAI after 3–6 years of treatment (median reservoir size, 1.1 IUPM; $P < .0005$). These results suggest that treatment <6 months after seroconversion may facilitate long-term control of cellular reservoirs that maintain HIV-1 infection during treatment.

Treatment of HIV-1 infection with highly active antiretroviral therapy (HAART) suppresses plasma viremia to <50 copies/mL in many patients. However, sensitive assays have demonstrated that CD4⁺ T cells retain replication-competent viral DNA and may be reactivated to produce virus even after years of viral suppression [1–3]. The elimination or control of this latent viral reservoir is thus an important goal in the refinement of long-term treatment strategies for HIV-1 infection

[4]. The CD4⁺ T cell reservoir is established during primary infection [5, 6]. However, the slow turnover of latently infected cells during chronic infection [7, 8] suggests that this reservoir might continue to fill over months to years and thus might be smaller during primary infection. One patient treated before serocon-

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version had no detectable cell-associated infectivity (CAI) [9], which is consistent with a limited reservoir size at the time of seroconversion.

The clearance kinetics of the latent virus population after initiation of HAART in patients during chronic infection remains controversial. In a cohort initiating therapy during chronic infection, Finzi et al. estimated the half-life of the CD4⁺ T cell latent reservoir to be 44 months [7]. Using a similar technique, Ramratnam et al. measured a half-life of 6 months in a cohort that included both chronically and acutely infected patients [8]. This shorter half-life suggests the possibility of eradication of HIV-1 from an infected patient by treatment with HAART. The discrepancy between these different measured clearance rates might be related to residual replication [8] or study duration [10], but it remains the subject of debate [11].

Although terminal dilution cocultures have been used to quantify cellular reservoirs of potentially infectious HIV-1, quantification of HIV-1 DNA level in peripheral blood mononuclear cells (PBMCs) provides a more sensitive alternate measure of reservoir size. Total HIV-1 DNA level has been shown to be stable throughout [12] and predictive of [13] disease progression, suggesting that it may be a useful marker for treatment decisions. After initial clearance of short- and long-lived productively infected cells [14], HIV-1 DNA is cleared with a half-life of 5–6 months in both chronic [14] and primary [15] infection. Whether the long-lived cells identified as containing HIV-1 DNA are dynamically related to the latent pool studied by quantitative coculture remains an important question.

The treatment of any patient identified with primary HIV-1 infection must be based on a rapid, individualized decision made on the basis of available information about potential long-term benefits and risks. Cumulative toxicity and the risk of developing or selecting for drug-resistant virus [16] argue against early treatment. Potential benefits to the patient include more-complete viral suppression [17] and long-term maintenance of HIV-1-specific CD4⁺ [18] and CD8⁺ [19, 20] T cell responses. Successful viral control has been observed when early treatment was followed by structured treatment interruptions in animal model studies [21, 22] and in humans [23, 24], although the duration of control varies. These results contrast notably with those of studies of therapy interruptions after treatment during chronic infection [25–28]. Thus, even if viral eradication by available therapies is not possible, there may nevertheless be significant benefits to early treatment.

A quantitative understanding of the dynamics of long-lived cellular reservoirs of HIV-1 in patients treated during primary HIV-1 infection may thus help refine long-term treatment regimens for HIV-1 infection. To assess the potential benefits of early treatment for the control of HIV-1 latency, we sought to extend previous results by comparing the size and clearance rate of the latent viral reservoir, as measured by both activated

coculture and HIV-1 DNA level, in patients initiating therapy at different stages of primary infection.

PATIENTS AND METHODS

Patient population. Patients were recruited to primary-infection studies in San Diego and Los Angeles between 1996 and 1999, on the basis of clinical symptoms or exposure history [29]. A subgroup of these patients volunteered to enroll in the present study. All participants gave written, informed consent, in accordance with the requirements of the local institutional review board. All 28 patients who elected to receive treatment and in whom viremia was suppressed to <50 copies/mL in plasma, as determined by the Roche Amplicor Ultrasensitive assay, were included in this analysis. They received continuous suppressive drug therapy for the duration of this study. In some patients in whom viremia had been suppressed to <50 copies/mL, the reappearance of low (>50 and <200 copies/mL) but detectable levels of viremia (“blips,” or intermittent viremia) occurred episodically, as has previously been reported in chronically infected patients [24]. All patients were male and had homosexual contact as a primary risk factor. Patients initiating therapy while results of an HIV-1 ELISA were negative and results of a Western blot were negative, indeterminate, or evolving (≤ 4 detectable bands) were designated as being “preseroconversion.” Patients initiating therapy later but while results of a detuned ELISA were still consistent with infection during the past 6 months (OD, <1.0) were defined as “postseroconversion.”

Fourteen patients who initiated HAART during chronic infection in clinical trial DMP266 [30] and 3 patients from clinical trial MRK035 [29] were also included, for cross-sectional comparison. These patients were also predominantly male but, as a group, had lower CD4⁺ T cell counts and plasma HIV-1 RNA levels at the time of treatment (median, 260 cells/mm³ and 4.78 log copies/mL, respectively).

HIV-1 DNA assays. PBMCs were extracted and then assayed for total HIV-1 DNA level by use of the Roche Monitor DNA assay [31]. Copy numbers per CD4⁺ T cell were computed using flow-activated cell (FAC) counts of the proportion of CD4⁺ T cells in a sample drawn simultaneously.

Coculture assays. Seventeen patients who agreed to donate the larger (80–160 mL) blood volumes necessary were prospectively enrolled in a protocol to quantify CAI. Recovery of virus from resting CD4⁺ T cells was accomplished by an ex vivo activation procedure [1, 32]. Either Rosette Sep (Stem Cell Technologies) or VarioMacs (Miltenyi-Biotec) was employed, in accordance with the manufacturers’ instructions, to isolate CD4-enriched patient T cells and CD8-depleted donor cells by negative selection. Cells were then stimulated with immobilized anti-CD3 (PharMingen) and interleukin (IL)–2. FAC analysis indicated that <2% of the remaining cells were CD8⁺ or CD14⁺, and, typically, >85% were CD4⁺. For each assay, CD8-depleted

cells pooled from at least 2 healthy control donors were activated with phytohemagglutinin (Sigma) and IL-2.

Cultures were performed with 1×10^4 – 3×10^6 cells/well in a 3-fold dilution series. Each cell dilution was assayed in quadruplicate. Fresh media and fresh activated donor PBMCs were added weekly, and supernatants were assayed for p24 by ELISA at days 7, 14, and 21. In 3 patients in whom no virus was detected in any well, 300 mL of whole blood was analyzed according to the above procedure, and 6–9 replicates of 3×10^6 cells were available. The assay sensitivity varied from 0.03 to 0.05 infectious units/million CD4⁺ T cells (IUPM) for these samples.

To assess the ability of this assay to reliably detect low levels of CAI, the same coculture procedure was used for 17 patients who had initiated HAART during chronic infection and in whom viremia had been suppressed to <50 copies/mL for 3–6 years. Dilution series used for these samples varied, but most included 24 replicates of 1×10^5 cells. The limit of detection in these assays was 0.17–0.40 IUPM.

Statistical methods. CAI was quantified as IUPM by use of a maximum-likelihood method, under an assumption of single-hit Poisson kinetics [33]. The sensitivity limit was defined as the maximum-likelihood estimate for an assay with 1 additional positive well at the lowest dilution (0.07 IUPM for a quadruplicate assay with wells containing 3×10^6 patient cells each). Viral reservoir sizes at 1 year were estimated by interpolating or extrapolating from available measurements, under an assumption of exponential decay after 4 weeks of treatment. When no decay rate could be measured in a given patient, the population median decay rate was assumed. Statistical comparison between groups, however, was based on a Mann-Whitney *U* test using only measured values (with undetectable values imputed as 0.07 IUPM). Total body viral reservoir sizes were estimated by assuming that peripheral blood CD4⁺ T cells were in equilibrium with 1.5×10^{11} total CD4⁺ T cells [34].

CAI clearance rates and reservoir sizes for individual patients were estimated using a maximum-likelihood fit under an assumption of log-normal errors. Clearance rates of HIV-1 DNA were estimated by least-squares minimization. Data before week 4 were excluded from all longitudinal analyses. Decay rates could be estimated in only 12 patients. In 5 patients, all in the preseroconversion cohort, CAI became undetectable by the first time point after 4 weeks, which prevented a quantitative rate estimate. Analyses were also performed with all data from before week 12 excluded; all group differences reported as significant were unaffected by this choice of start time. HIV-1 DNA clearance rates during the first year and subsequent years were compared using a paired Wilcoxon test. Correlations were computed using the Pearson correlation coefficient, and their significance was tested using a Wilcoxon rank-sum test.

RESULTS

Baseline characteristics of the patient population. Of 40 original study subjects, 30 consented to high-volume blood draws for CAI studies, whereas 10 provided lower-volume blood donations suitable only for HIV-1 DNA measurements. An additional 14 patients were subsequently excluded from the study, either because they did not initiate HAART or sustain suppression ($n = 10$) or because of insufficient follow-up ($n = 4$).

Baseline characteristics of the patients who finally qualified for the study are given in table 1. There were no significant differences in pretreatment CD4⁺ T cell counts, HIV-1 DNA copy number per microgram of PBMC DNA, or CAI between the preseroconversion and postseroconversion cohorts ($P > .5$ in all cases), nor was there a major difference in the proportion of patients exhibiting intermittent viremia between the 2 cohorts (5/13 in the preseroconversion cohort; 6/14 in the postseroconversion cohort). The frequency of intermittent viremia in these patients was in line with those reported in the study of chronically infected patients receiving HAART [35]. The median pretreatment HIV-1 RNA level was higher in patients treated before seroconversion (>500,000 copies/mL) than in patients treated after seroconversion (190,000 copies/mL), but the baseline viral load did not differ significantly between cohorts ($P = .31$).

CAI clearance during the first year of HAART. CAI decayed rapidly during the first month of therapy, as reported elsewhere [36]. Decay of CAI was therefore measured beginning at week 4 of treatment, before which the majority of both productively infected cells and any cells in a preintegration form of latency would have cleared. The median half-lives in the preseroconversion and postseroconversion cohorts were 14 weeks and 20 weeks, respectively; this difference did not approach statistical significance ($P = .8$). The median half-life in the combined primary-infection cohort after week 4 of HAART was 20 weeks; excluding all data collected before week 12 did not affect this estimate (figure 1).

HIV-1 DNA clearance during the first year of HAART. HIV-1 DNA assays were performed longitudinally on 23 patients (table 1). In 8 of these patients, banked PBMC samples were analyzed retrospectively for 3–4 years after initiation of HAART. During the first year, HIV-1 DNA decayed with a median half-life of 30 weeks (range, 10–110 weeks; figure 2). Because CD4⁺ T cell counts recovered substantially during the first year of treatment, the clearance of HIV-1 DNA per CD4⁺ T cell was significantly faster than clearance per PBMC. The median half-life of HIV-1 DNA in this normalization was 18 weeks in both treatment cohorts (table 2).

Nonlinear kinetics of HIV-1 DNA clearance. Analysis of the 9 patients with longer follow-up demonstrated that decay of HIV-1 DNA was not simply exponential during the 4-year treatment period (figure 3). In the 8 patients followed after the first year,

Table 1. Summary of baseline patient characteristics.

Time of initiation of treatment, patient ID	Baseline CD4 ⁺ T cell count, cells/ μ L	Baseline log ₁₀ HIV-1 RNA level, copies/mL	Baseline HIV-1 DNA level, copies/ μ g	Baseline coculture infectivity, IUPM	HAART regimen	Blip
Before seroconversion						
001	687	>5.7	NA	110	D4T, 3TC, ABC, NVP, HU	No
002	45	>5.7	NA	45	D4T, DDI, NFV	Yes
004	847	5.3	34	NA	AZT, 3TC, IDV	No
009	468	5.2	NA	110	D4T, 3TC, NFV	Yes
017	1521	4.0	15	3.2	D4T, 3TC, APV	No
019	185	>5.7	144	2.02	D4T, 3TC, NFV	No
020	497	>5.7	298	37.3	AZT, 3TC, ABC	No
021	637	4.2	171	13.7	D4T, 3TC, ABC, NVP	Yes
044	408	>5.7	355	NA	AZT, 3TC, NFV	No
045	291	5.3	547	NA	ABC, APV	Yes
056	909	3.7	120	NA	EFV, IDV	Yes
178	474	4.2	1466	15.7	3TC, D4T, ABC, RTV, APV	No
404	1031	>5.7	NA	57	AZT, 3TC, NFV	No
After seroconversion						
003	605	5.0	60	NA	AZT, 3TC, IDV	No
013	304	5.3	NA	110	AZT, 3TC, ABC	No
022	785	>5.7	NA	NA	D4T, 3TC, LPV, RTV	Yes
031	1048	>5.7	17	NA	AZT, ddC, LPV, RTV	Yes
054	427	>5.7	188	NA	AZT, 3TC, NFV	Yes
063	344	5.1	236	NA	3TC, D4T, NFV	No
065	305	5.3	2439	NA	AZT, 3TC, NFV	Yes
118	593	4.7	124	110	DDI, D4T, EFV, NFV	Yes
122	348	5.0	247	44	AZT, 3TC, ABC, APV	No
125	426	5.3	451	1.1	3TC, AZT, ABC, APV	Yes
133	230	3.6	1422	NA	AZT, 3TC, ABC, APV	No
135	542	>5.7	65	1.57	AZT, 3TC, RTV, IDV	No
143	596	5.5	233	9.5	DDI, D4T, EFV, NFV	No
185	528	>5.7	47	NA	3TC, D4T, ABC, RTV, APV	No
Median (all patients)	486	5.3	180	16		
Median (before seroconversion)	497	>5.7	157	16		
Median (after seroconversion)	468	5.3	211	27		

NOTE. ABC, abacavir; APV, amprenavir; AZT, zidovudine; ddC, zalcitabine; DDI, didanosine; D4T, stavudine; EFV, efavirenz; HAART, highly active antiretroviral therapy; HU, hydroxyurea; IDV, indinavir; IUPM, infectious units per million cells; LPV, lopinavir; NA, not available; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir; 3TC, lamivudine.

the median decay of HIV-1 DNA per unit of PBMC DNA was -0.0093 per week (range, -0.024 to 0.0005 per week), corresponding to a half-life of 70 weeks. The median decay of HIV-1 DNA per CD4⁺ T cell was -0.012 per week (range, -0.026 to -0.0002 per week), corresponding to a half-life of 60 weeks. In both normalizations, DNA clearance was significantly slower ($P < .001$) after the first year than between weeks 4 and 48 of treatment. Thus, DNA decay was estimated independently for the first year (weeks 4–48) and for subsequent years.

Residual cellular reservoirs of HIV-1. The total size of the latent CD4⁺ T cell reservoir after 1 year of treatment could not be directly measured in the primary-infection cohort. Specifically, virus eventually could no longer be recovered from any of the 9 patients who were treated before seroconversion. The treatment duration at which CAI fell below the assay threshold varied from 4 to 62 weeks. By direct measurement, we found

that the reservoir size was <0.07 IUPM in all patients who initiated HAART before seroconversion and in 6 of 8 patients who initiated HAART <6 months after seroconversion. Extrapolated estimates of the reservoir size indicated median reservoir sizes of 0.03 IUPM and 0.09 IUPM, in the pre- and postseroconversion cohorts, respectively.

Among 36 samples taken from 17 chronically infected patients after 3–6 years of virologic suppression, the same activation protocol resulted in virus detection in 30 samples, despite examination of typically fewer cells from these patients. At least 1 sample from each patient retained detectable CAI. The median CAI was 1.1 IUPM, which was significantly higher than in patients treated before ($P = .00002$) or <6 months after ($P = .0005$) seroconversion.

The total number of replication-competent latently infected cells was also estimated for each patient (figure 4). For patients

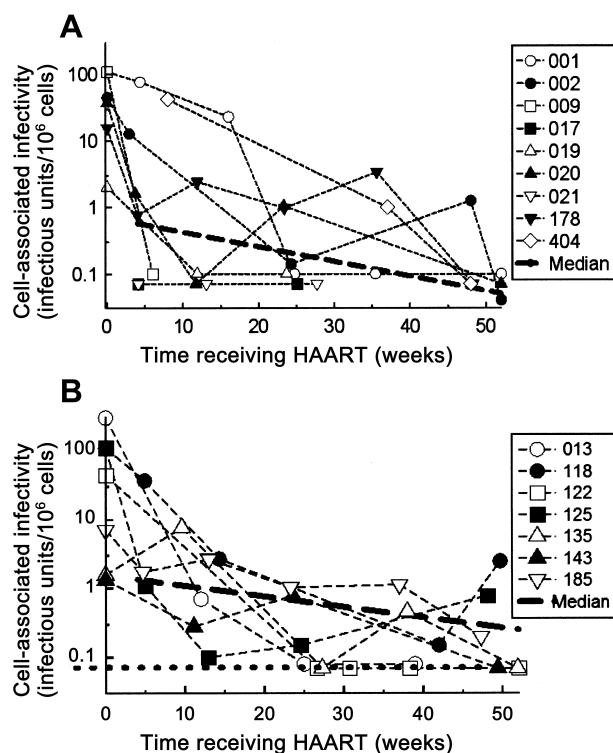


Figure 1. Decay of cell-associated infectivity (CAI). After an initial rapid decline during the first month of highly active antiretroviral therapy (HAART), CAI decayed with a median half-life of 14 weeks in patients who initiated HAART before seroconversion (A) and 20 weeks in patients who initiated HAART <6 months after complete seroconversion (B). By the end of the first year of treatment, CAI had fallen below the level of detection (red dashed line) in 12 patients, including all 9 acutely infected patients shown in panel A. Black dashed lines show the median decay slope and median residual CAI (table 2).

in the pre- and postseroconversion cohorts, reservoir sizes after 48 weeks of HAART were estimated as described in Patients and Methods. Reservoir sizes in patients who initiated treatment during chronic infection were estimated at the time of collection of available samples, after 3–6 years of HAART. The median estimated reservoir sizes were 4000 cells for patients who initiated therapy before seroconversion, 13,000 cells for patients who initiated therapy <6 months after seroconversion, and 160,000 cells for chronically infected patients.

DISCUSSION

Sustained control of cellular reservoirs of HIV-1 remains an important goal in the development of antiretroviral therapies. Although estimates of the duration of HAART that is required to eradicate the latently infected cell population from chronically infected patients extend into decades [7], treatment early during infection might limit the establishment or alter the clearance kinetics of this population. We investigated both of these

hypotheses by longitudinally following patients initiating therapy before and soon after seroconversion.

After 1 year of treatment, replication-competent virus could not be detected (<0.07 IUPM) in 9 of 9 patients who initiated HAART before seroconversion or in 6 of 8 patients who initiated HAART <6 months after seroconversion. We assume that the lack of recovery of viable virus did not indicate the absence of latently infected cells in vivo but reflected their relative infrequency in these patients and blood-volume limits. Two patients who initiated therapy during early infection harbored intermediate numbers of latently infected cells, which is consistent with previous reports of detectable cellular reservoirs in patients treated during primary infection [5, 9]. Larger reservoirs (median, 1.1 IUPM), consistent with previous measurements [6, 7], were observed in patients treated for an average of 6 years after therapy initiation during chronic HIV-1 infec-

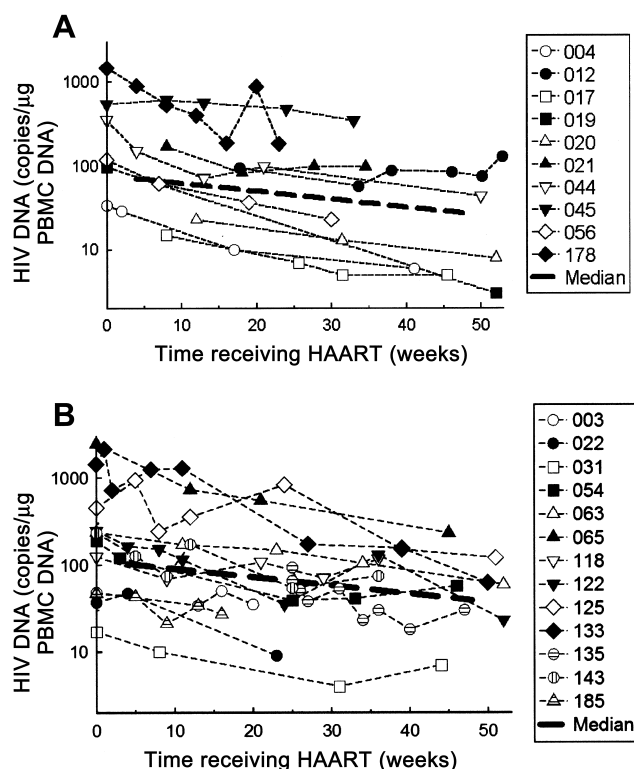


Figure 2. Decay of HIV-1 DNA during the first year of highly active antiretroviral therapy (HAART). During the first year of HAART, HIV-1 DNA in peripheral blood mononuclear cells (PBMCs) was cleared with a median half-life of 30 weeks, with no significant difference between patients initiating treatment before (A) or after (B) seroconversion. This estimated half-life is in line with published estimates for both chronic [14] and acute [15] infection. When normalized to CD4⁺ T cell counts, HIV-1 DNA clearance is faster (median half-life, 18 weeks) because of CD4⁺ T cell recovery on HAART. After initial clearance of labile molecular forms of HIV-1, the kinetics of DNA in this latter normalization were indistinguishable from the kinetics of infectivity (figure 1), suggesting that viral DNA dynamics parallel the dynamics of replication-competent virus.

Table 2. Summary of cellular reservoir size and clearance characteristics.

Time of initiation of treatment, patient ID	Baseline HIV-1 DNA level, copies/ μ g	Decay constant (per week)			Residual size at week 48	
		HIV-1 DNA	HIV-1 DNA/ CD4 ⁺ T cell	Infectivity	Infectivity, IUPM	HIV-1 DNA level, copies/ μ g
Before seroconversion						
001	−0.12	0.03	...
002	0.0003	0.106	...
004	34	−0.0421	−0.0374	4
009	ND	0.015	...
017	15	−0.0308	−0.0484	ND	0.015	4
019	144	0.01183	0.02	10
020	298	−0.02177	−0.03	−0.033	0.114	11
021	171	−0.0178	−0.0293	ND	0.015	68
044	355	−0.0231	−0.0258	42
045	547	−0.0209	−0.0387	269
056	120	−0.0421	−0.0493	11
178	1466	−0.05219	−0.0812	−0.069	0.07	71
404	−0.151	0.07	...
After seroconversion						
003	60	−0.0248	−0.0244	ND	...	18
013	−0.17	0.031	...
022	...	−0.06899	...	ND	0.015	4
031	17	−0.021	−0.0423	5
054	188	−0.0191	−0.0241	38
063	236	−0.0125	−0.0119	82
065	2439	−0.0354	−0.0435	205
118	124	0.013406	−0.0063	−0.139	0.08	120
122	247	−0.0183	−0.0381	−0.011	0.034	37
125	451	−0.027817	−0.0404	0.0148	0.78	151
133	1422	−0.07486	−0.0772	67
135	65	−0.04975	−0.1105	−0.047	0.25	18
143	233	−0.019398	−0.0239	−0.035	0.07	51
185	47	−0.0231	−0.0726	−0.031	0.34	13
Median (all patients)	180	−0.022	−0.038	−0.035	0.033	38
Median (before seroconversion)	211	−0.022	−0.039	−0.035	0.087	38
Median (after seroconversion)	157	−0.022	−0.037	−0.051	0.025	27

NOTE. Ellipses (...) indicate that no sample was available. IUPM, infectious units per million cells; ND, not determined because ≤ 2 cocultures after week 4 contained detectable infectivity.

tion. If we assume that proportionality between peripheral blood T cells and tissue T cells [37] is established early during infection [38], these measurements suggest a median of only 5000 latently infected CD4⁺ T cells in patients initiating HAART <6 months after seroconversion. In chronically infected patients, this median was >160,000 cells after several years of treatment, which is consistent with previous estimates [6, 7]. These results demonstrate that early treatment limits the size of the latent reservoir, as suggested by a previous cross-sectional study [9].

The first year of treatment resulted in a significant clearance of cellular reservoirs of HIV-1. During primary HIV-1 infection, patients may have a greater proportion of infected cells responding specifically to HIV-1 antigens [39]; these cells would encounter their cognate antigens frequently, resulting in rapid cell turnover. Previous studies have also demonstrated an elevated state of immune activation in primary HIV-1 infection

[40–43], suggesting that cell clearance might be enhanced by nonspecific activation. Alternatively, immune clearance of infected cells by HIV-1–specific cytotoxic T lymphocytes (CTLs), which are preserved by early initiation of HAART [18, 20], might more effectively contribute to clearance. Finally, the smaller observed reservoir sizes in patients treated during primary infection could represent a more profound inhibition of viral replication [45, 53] in such patients, as is supported by one study of residual viremia in patients treated during primary infection [57]. However the proportion of patients with intermittent viremia (“blips”)—a surrogate for residual viral replication—who started treatment before and after seroconversion was no different from proportions reported in studies of chronically infected patients [24].

Because CAI was undetectable by the end of the first year in a majority of patients with primary infection, subsequent

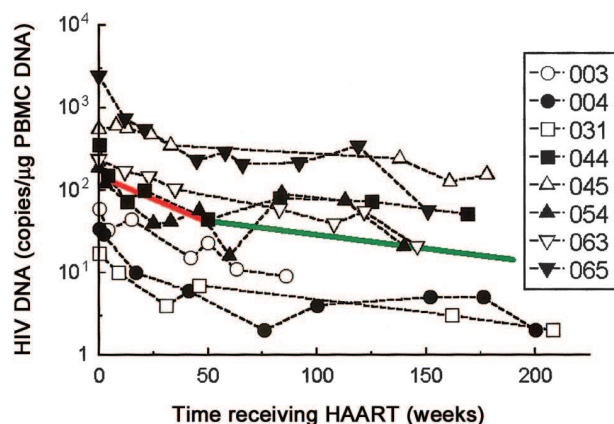


Figure 3. Slower-than-exponential decay of HIV-1 DNA during the first 4 years of highly active antiretroviral therapy (HAART). Clearance during the first year occurred with a median half-life of 30 weeks in both the preseroconversion (figure 2A) and postseroconversion (figure 2B) cohorts. At the end of the first year, HIV-1 DNA was not significantly different between the 2 groups. A subgroup of 8 patients with longer follow-up is shown above. The decay kinetics during the first year (red) are statistically indistinguishable from those of the entire cohort. During the 4-year period shown, clearance of HIV-1 DNA was not simply exponential, with significantly slower clearance ($P = .001$) during the subsequent 3 years (green) of suppressive therapy (median half-life, 70 weeks). These nonlinear kinetics might be due to heterogeneity of cellular reservoirs [10], so that clearance progressively selects for the most-stable latent cells.

quantification was based on HIV-1 DNA level, a more sensitive measure of the population size of cellular reservoirs. The relationship between HIV-1 DNA level and CAI, both of which are measures of the size of the cellular reservoir of HIV-1, is not well understood. During antiretroviral therapy, the majority of HIV-1 DNA in infected cells is integrated into the host genome [44]. However, unintegrated linear and circular forms of HIV-1 DNA are also present. Although the former are short lived, the latter may be very stable [45–47] but represent a small minority of the total HIV-1 DNA [9].

Our results provide empirical evidence that HIV-1 DNA level can serve as a useful measure of the dynamics of CAI after the initial month of therapy. We observed a rapid decline in CAI during the first month of treatment, with no parallel decline in HIV-1 DNA level (figure 1). This is consistent with the existence of a subset of cells with labile, unintegrated, full-length HIV-1 DNA, previously estimated to have a functional half-life of 6 days [36], provided that these unintegrated molecular forms are, on average, more likely to give rise to productive infection than are integrated HIV-1 genomes during the period immediately after initiation of suppressive therapy. Because our coculture assays were performed on CD4-enriched cells, the clearance rates of HIV-1 DNA per CD4⁺ T cell were computed for comparison. During weeks 4–48 of HAART, the median decay half-lives of HIV-1 DNA and CAI were the same (20 weeks). The parallel

kinetics of HIV-1 DNA and CAI suggest that HIV-1 DNA level may provide a convenient marker of the cellular reservoir of virus after approximately the fourth week of treatment and that the proportion of HIV-1 DNA genomes that are replication competent remains stable through this period.

Previous studies of the dynamics of HIV-1 latency have been performed under the assumption that the clearance of this reservoir was exponential [7, 8]. However, we found that the clearance rates of HIV-1 DNA declined substantially after the first year of treatment. This is consistent with the findings of a study reporting that cellular reservoirs of HIV-1 contain cells that clear at different rates [10]. In 8 patients followed for 3–4 years, the median half-life of DNA (per CD4⁺ T cell) after the first year of HAART was 60 weeks, which was statistically different ($P = .003$) from the 20-week half-life observed in the same patients during the first year. This difference did not reflect a change in the degree of viral suppression, since all patients retained plasma HIV-1 RNA levels <50 copies/mL throughout the study. This range of values is consistent with those from previous studies of both chronic and primary infection [6, 14, 15]. The change in clearance kinetics might reflect decreasing activation rates after viral suppression [48]. Alternatively, cells

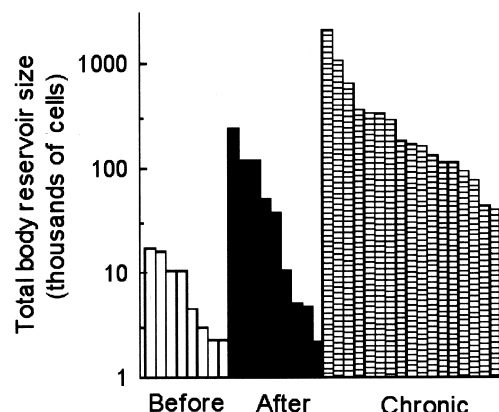


Figure 4. Attenuation of residual cellular reservoirs of culturable HIV-1 by initiation of highly active antiretroviral therapy (HAART) during primary infection. Patients treated either before or <6 months after seroconversion harbored smaller reservoirs of recoverable HIV-1 after 1 year of HAART than did patients treated with HAART for 3–6 years after initial treatment during chronic infection. In samples from the majority of patients with primary infection (9/9 in the preseroconversion cohort and 6/8 in the postseroconversion cohort), infectious virus could no longer be cultured by the end of the study period; the values shown were extrapolated to week 48 of HAART, by use of the population median clearance. Replication-competent virus was recovered from all 17 chronically infected control individuals shown, despite a less sensitive assay and 2–5 additional years of suppressive HAART (chronic vs. preseroconversion, $P = .00002$; chronic vs. postseroconversion, $P = .0005$). Thus, establishment of long-lived cellular reservoirs of HIV-1 begins before seroconversion but occurs predominantly >6 months after seroconversion.

recognizing common antigens may be preferentially activated and cleared during the first year, leaving latently infected memory cells that recognize rarely encountered antigens to activate slowly in subsequent years [10, 49]. The different dynamics of phenotypically distinct populations of long-lived cells, including memory T cells, naive T cells [50], thymocytes, and other cell types [51–53], might also contribute to the decline in clearance.

Although our results do not address the mechanism underlying changing HIV-1 DNA clearance rates, they suggest that the rapid clearance of CAI during the first year may not be sustained thereafter. If CAI kinetics continues to parallel that of HIV-1 DNA at the rate observed after the first year, spontaneous clearance of the reservoir would require ~15 years of HAART, even for patients initiating HAART before seroconversion. The observation that DNA clearance decreases during the first few years of HAART, however, suggests the possibility that this deceleration continues, which is consistent with dynamically heterogeneous cellular reservoirs being present [10]. Progressively decelerating clearance [49] might explain why previous studies of chronic infection have found variable half-lives [7, 8], with longer half-lives associated with longer follow-up [54]. If clearance continues to slow through subsequent years of infection, even the small cellular reservoirs we observed would not be eradicated in a patient's lifetime. If, as has been proposed, the latent reservoir is responsible for viral rebound during therapy interruption [25], this small reservoir size may explain the delayed rebound during interruptions in patients with primary infection [24, 55].

Although eradication of infection appears unachievable with currently available treatment, the difference in reservoir characteristics for patients treated early during primary infection may nonetheless be significant. Novel strategies designed to mobilize virus from resting CD4⁺ T cells [56] or immune modulation strategies, such as therapeutic vaccination [57], may have the greatest likelihood of success in patients treated early enough to both limit the extent of cellular reservoirs and preserve antiviral immune function. The high degree of suppression and limitation of reservoir size seen in this primary-infection cohort may enhance the long-term durability of treatment responses, since higher levels of viral production have been reported to result in the acquisition of some drug-resistance mutations, even in patients generally responding to potent therapy [59]. Latently infected cells may be an important source of residual infectious virus during sustained [58] and interrupted treatment. Thus, smaller reservoirs may facilitate the use of simplified maintenance regimens [59] or planned treatment interruptions, reducing the toxicity and cost of long-term antiretroviral therapy. Thus, initiation of HAART during primary infection may increase patients' future options for long-term control of HIV-1 infection.

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